Conductin Protein and a Related Agent For Diagnosing and Treating Tumor Illnesses

Field of the invention

The invention relates to a new way of combating tumor diseases by utilizing molecular biological relationships of the formation of tumors. In particular, it relates to a new agent for diagnosing tumor diseases and a material based as the new agent for the treatment of such diseases. The invention also relates to the new protein conductin, its mutants and variations as well as to parts thereof, to the analogous cDNA sequences and to their use in the gene-therapy and pharmacological methods.

Background

Cadherines and catenines form cell adhesion complexes, which are responsible in numerous tissues for the adhesion of cells to one another. The cadherines are trans-membrane proteins and produce the direct contact between adjacent cells. α-, ß- and ɣ-catenine are cytoplasmic components, which connect the cadherines with the actin cytoskeleton. Aside from their function in cell adhesion, the catenines also play a decisive role in signal transduction processes. ß-Catenine in vertebrates and the homologous, segment polarity gene product, armadillo in drosophila, are stabilized by the Wnt/wingless signal path (Nusse, R., Cell 89, 321 – 323, 1997). This leads to an increase in the cytoplasmic fraction of these proteins, which is not bound to cadherine, which thereupon could interact with HMG transcription factors of the LEF-1/TCF families. As a result, ß-catenine/armadillo is transported into the cell nucleus where, together with the LEF/TCF proteins, it binds

to the DNA and activates certain genes (Behrens, J. et al., Nature 382, 638 – 642, 1996).

This signal path also plays an important role in the formation of tumors. In epithelial cells of the colon, the cytoplasmic pool of ß-catenine is strictly regulated by the tumor suppressor gene product APC (Adenomatosis Polyposis Coli). Mutations of APC, such as those occurring in 80% of all colon cancers, lead to shortened forms of the APC protein, which are no longer able to destabilize ß-catenine. As a result, permanent complexes of ß-catenine with the HMG transcription factor TCF-4, which are asserted to be responsible for the transformation of the cells, are found in these tumors. This theory is supported by the recent finding that, in tumors in which APC is not changed, mutations of ß-catenine occur. These also lead to cytoplasmic stabilization of ß-catenine and to an association with LEF-1/TCF factors (Morin, P.J. et al., Science 275, 1787 – 1790).

Brief description of the drawing

The invention is disclosed below with reference being had to the drawing, wherein

Fig. 1 is the amino acid sequence 1 to 840 of conductine;

Fig. 2 is the nucleotide sequence of conductine;

Fig. 3 is the gene comparison sequence and the nucleotide sequence; and

Fig. 4 is a showing of of interaction stuidies in the 2-hybrid system.

Description of the invention

It is an object of the present invention to find a new way to prevent the formation of tumors. It is based on the objective of finding a method for controlling the regulation of ß-catenine in cells of the body.

It is an object of the present invention to identify a new protein which binds to ß-catenine and leads to its cytoplasmic breakdown. This protein has the amino acid sequence shown in Fig. 1 and we gave it the name conductin.

The invention is based on our realization that conductin binds to APC fragments over a ß-catenine binding domain at ß-catenine, over a glycogen synthase kinase 3ß (GSK 3b) binding domain at GSK 3b and over a so-called domain signaling regulator of G-protein (RGS) which is a part of conductin and interacts with the tumor suppressor protein APC. As a result, there is cytoplasmic degradation of ß-catenine and in vertebrates, blockage of the Wnt/wingless signal path. That establishes that conductine is an important regulator of the ß-catenine function and in interaction with APC contributes to the suppression of tumors.

Thus the invention relates to a material for diagnosing tumor diseases, which is characterized by the presence and the amount of conductine, its mutants and variations or its parts detected in cells of the body. This detection can be carried out on the protein level with specific antibodies, particularly with monoclonal antibodies.

Pursuant to the present invention, the diagnosis of tumor diseases can also be carried out on the gene level. For this purpose,

- the gene, which codes for conductine, its mutants and variations or parts thereof and/or
- mRNA sequences, which are read by these genes,

are detected with selected oligonucleotide primers and cDNA probes, which are derived from the gene sequence of the conductine and inutations.

The material of the present invention for the treatment of tumor diseases contains substances which activate/reactivate the action of conductin in the body. Above all, these are materials, which activate the gene promoter of conductin or materials, which increase the stability of the mRNA sequences derived from the conductin genes. Pursuant to the invention, the main objective of all of these materials to increase the activity of the conductin in the cells of the body. For this purpose, for example, low molecular weight substances, come into consideration, which are found, for example, by high throughput number screening. High throughput screening can be performed by analyzing low molecular weight substances for their ability to stimulate the activity of the conductin promoter or the expression of the conductin mRNA protein after treatment of cultured cells. Alternatively, substances can be screened for active phospharylation of ß-catenine by conductin in *in vitro* kinase reactions.

The present invention also includes gene therapeutic materials, containing genes, which code for conductin, its mutants and variations or parts thereof, or mRNA sequences, which are read by these genes.

Furthermore, the new protein conductin of Fig. 1 - SEQ ID No. 1 its mutants and variations, as well as parts thereof are a part of the present invention. Especially suitable partial sequences are the amino acids 78 to 200 (RGS) – SEQ ID No. 2, 343 - 396 (GSK 3b-binding domains) – SEQ ID. No. 3, 397 - 465 (b-catenine binding domains) – SEQ ID No. 4 and 783 - 833 (disheveled homology region) – SEQ ID No. 5. Partial sequences of the Adenomatosis Poliposis Coli (APC), which are characterized by the amino acid sequences 1464 – 1604, 1516 – 1595, 1690 – 1778 and 1995 – 2083 as RGS-domain interaction sites, are also part of the extent of the invention, as are also the analogous cDNA sequences, especially the full cDNA sequence of the conductin (base pairs 1 – 2825) of Fig. 2 – SEQ ID No. 6, as well as

the partial sequences of the conductin of the nucleotide sequence 446 – 814 (RGS gene section) – SEQ ID No. 7, of the nucleotide sequence 1241 – 1402 (gene section of GSK 3b-binding domains) – SEQ ID No. 8, 1403 – 1609 (gene section of the ß-catenine binding domains) – SEQ ID No. 9 and of the nucleotide sequence 2561 – 2713 (gene section of the disheveled homology region) – SEQ ID No. 10.

The present invention also relates to a gene therapy process for tumor diseases, which comprises constructing a vector with the conductine gene and restoring conductine in cells of a patient in need therefor by carrying out a gene transfer in the body of the patient

The invention is explained in greater detail by reference to the following examples.

Conductin was identified by a yeast 2-hybrid screen as a ß-catenine interaction partner. The complete cDNA sequence was subsequently isolated and sequenced. The derived amino acid sequence of conductin is shown in Fig. 1. The nucleotide sequence is shown in Fig. 2 at Position 1-2825 and the gene comparison of the amino acid sequence and the nucleotide sequence is shown in Fig. 3 with the sequence regions marked as in Fig. 1. The conductin cDNA codes a protein of 840 amino acids and has a calculated molecular weight of 92.8 kDa. The RGS domains (shown in double underlining), the ß-catenine binding domains (shown in simple underling), and the disheveled homology region (bold letters) are emphasized. By a comparison of sequences, an RGS domain (amino acid 78 – 200) and a domain (amino acid 783 – 833, disheveled homology region), related to the protein disheveled, were identified (Figs. 1 – 3). The GSK 3b- and ß-catenine binding domains (amino acids 343 – 396 to 397 – 465) were discovered by interaction studies in the 2-hybrid system (Fig. 4). It was observed that these domains are

sufficient and necessary for the binding to GSK 3b or to ß-catenine (Fig. 4). The conductine protein and derived partial pieces are shown diagrammatically. The RGS domains (RGS), the GSK 3b-binding domains (GSK BD) and the ß-catenine binding sites (b-BD) are emphasized. The interaction with ß-catenine with the APC fragments of amino acids 1464 – 1604 (APCfr.1) and 1516 – 1595 (APCfr. 2) and GSK 3b were investigated in the yeast 2-hybrid assay and quantified as ß-galactosidase units. It can be seen that the binding of the ß-catenine to the ß-catenine binding site is limited; the other parts of the protein do not contribute to this. The analysis furthermore shows the exclusive interaction of APC with the RGS domains of conductin. Comparable results for the binding to the RGS domains were obtained with the APC fragments of amino acids 1690 – 1778 and 1995 – 2083. The breakdown of ß-catenine into SW480 cells by conductin was analyzed after transient expression of the given proteins and immunofluorescence staining of ß-catenine. Only partial pieces of conductin, which bind to ß-catenine, lead to this breakdown. The analysis finally shows the binding of GSK 3b to the GSK 3b-binding domains of conductin. On the other hand, the RGS homology region and the disheveled homology region do not participate. The interaction of conductine with GSK 3b and ß-catenine was also biochemically confirmed in co-immunoprecipitation experiments.

The effect of conductin on ß-catenine was investigated in SW480 cells. In these cells, the tumor suppressor gene product APC is mutated, as a result of which there is an increase in the cytoplasmic and especially in the nuclear content of ß-catenine. The introduction of conductin into these cells leads to a drastic breakdown of ß-catenin, as a result of which the cells are depleted of cytoplasmic ß-catenine and of ß-catenine in the cell nucleus (Fig. 4). This effect on the content of ß-catenine is equal in intensity to that of not-mutated APC, from which it can be concluded that conductin also acts as a tumor suppressor by regulating ß-catenin.

Moreover, it was shown that conductin also inhibits the Wnt/wingless signal path in Xenopus embryos due to its effect on b-catenine. Wnt/wingless are secreted proteins that counteract conductin function in various tissues.

It was also noted that conductin interacts directly with APC. APC fragments of amino acids 1464 – 1604, 1516 – 1595, 1690 – 1778 and 1995 – 2083 were identified as interaction sites for conductin. In conductin, the binding to APC takes place over the RGS domains; this region is sufficient and necessary for the interaction. The other domains in conductin do not participate (Fig. 4).